

## CLAIMS

- 1/ Sequence of nucleotides coding for at least a part of the N-terminal region of a polypeptide specifically toxic towards larvae of the Lepidoptera of the family of the Noctuidae, and preferably towards S.littoralis, characterized by its capacity of hybridization with a gene capable of expressing a polypeptide toxic towards larvae of S.littoralis.
- 2/ Sequence of nucleotides of about 3 kb corresponding to the HindIII-PstI restriction fragment derived from B.thuringiensis capable of hybridizing with the probes 1, 2, 3 of pHTA2 reported in figure 2.
- 3/ Sequence according to Claim 1 or 2, characterized in that it contains in the following order the sites :  
HindIII - HincII - BglIII - KpnI - HindIII - PstI.
- 4/ Sequence of nucleotides according to any one of the Claims 1 to 3, characterized in that it is obtained in vitro from a single strain of B.thuringiensis.
- 5/ Sequence of nucleotides according to Claim 4, characterized in that the strain of B.thuringiensis is the aizawai 7-29 strain.
- 6/ Sequence of nucleotides according to any one of the Claims 1 to 3, characterized in that it is obtained by in vitro genetic recombination of DNA sequences from two different strains of B.thuringiensis.
- 7/ Sequence according to Claim 6, characterized in that the 2 strains of B.thuringiensis correspond to the strains entomocidus 6-01 and aizawai 7-29, respectively.
- 8/ Sequence of nucleotides, characterized in that it codes for a polypeptide capable of forming an immunological complex with antibodies directed against polypeptides with a larvicidal activity towards S.littoralis.
- 9/ Sequence of nucleotides characterized in that it has the capacity to hybridize with a probe formed from the sequence (I) exhibiting the following chain arrangement :

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 GTC TAC TTG ACA GCG GTA GGA ACA TAA TCG GTC AAT TTT AAA TAT GCG GCA TAT ATT GAT  
 112  
 ATT TTA TAA AAT TTG TTA CGT TTT TTG TAT TTT TTC ATA ACA TGT CTC ATA TGT ATT AAA  
 172  
 TCG TCG TAA TGA AAA ACA GTA TCA AAC TAT CAG AAC TTT GGT AGT TTA ATA AAA AAA CCG  
 232  
 AGG TAT TTT ATG GAG GAA AAT AAT CAA AAT CAA TCG ATA CCT TAC AAT TGT TTA AGT AAT  
 292  
 CCT GAA GAA GTA CTT TTG GAT CGA GAA CCG ATA TCA ACT GGT AAT TCA TCA ATT GAT ATT  
 352  
 TCT CTG TCA CTT GTT CAG TTT CTG GTA TCT AAC TTT GTA CCA GCG GGA GCA TTT TTA GTT  
 412  
 CGA TTA ATA GAT TTT GTA TCG GGA ATA GTT GCG CCT TCT CAA TCG GAT GCA TTT CTA GTA  
 472  
 CAA ATT GAA CAA TTA ATT AAT GAA AGA ATA GCT GAA TTT GGT AGG AAT GGT GGT ATT GGT  
 532  
 AAT TTA GAA GGA TTA GGA AAC AAT TTC AAT ATA TAT GTC GAA GCA TTT AAA CAA TCG GAA  
 592  
 GAA GAT CCT AAT AAT CCA GAA ACC AGG ACC AGA GTA ATT GAT CCG TTT GGT ATA CTT GAT  
 652  
 GCG CTA CTT GAA AGG GAC ATT CCT TCG TTT CGA ATT TCT GGA TTT GAA GTA CCC CTT TTA  
 712  
 TCG GTT TAT GGT CAA GCG GCG AAT CTG CAT CTA GGT ATA TTA AGA GAT TCT GTA ATT TTT  
 772  
 CGA GAA AGA TCG GGA TTG ACA ACG ATA AAT GTC AAT GAA AAC TAT AAT AGA CTA ATT AGG  
 832  
 CAT ATT GAT GAA TAT GGT GAT CAC TGT GCA AAT ACG TAT AAT CCG GGA TTA AAT AAT TTA  
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 CCC AAA TCT ACG TAT CAA GAT TCG ATA ACA TAT AAT CGA TTA CCG AGA GAC TTA ACA TTG  
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or from the sequence (III) exhibiting the following chain arrangement :

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10/ Sequence of nucleotides coding for a polypeptide toxic specifically towards larvae of the Lepidoptera of the family of the Noctuidae, and preferably towards S.littoralis, characterized in that it includes the chain arrangement (I) or (III) defined in Claim 9.

5 11/ Sequence of nucleotides according to Claim 9 or 10, characterized in that it has an ATG initiation codon situated at position 241.

12/ Sequence according to any one of the Claims 9 to 11, characterized by a GGAGG binding site to ribosomes at positions 230 to 234.

10 13/ Sequence according to one of the Claims 10 to 12, characterized in that it contains the sequence included between the nucleotides at position 137 and 177 (position -103 to -63) upstream from the ATG initiation codon) which is homologous to the extent of about at least 70% with the region present upstream from the gene for the crystal of the strain kurstaki-HD1 Dipel (BTK) which contains the three promoters BtI, BtII and Ec, functional in B.thuringiensis and E.coli, respectively.

15 14/ Sequence of nucleotides, characterized in that it codes for a polypeptide comprising the sequence of amino acids (II) below :

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00445 "00445" 00445

or that it codes for a polypeptide comprising the sequence of amino acids (IV) below:

101030" 58431660

W O 9812

- 44 -

PCT/H R88/00292

271 MET GLU GLU ASN ASN GLN ASN GLN LYS ILE  
PRO TYR ASN CYS LEU SER ASN PRO GLU GLU VAL LEU LEU ASP GLY GLU ARG ILE SER THR GLY ASN SER SER ILE ASP ILE SER LEU SER  
361 LEU VAL GLN PHE LEU VAL SER ASN PHE VAL PRO GLY GLY PHE LEU VAL GLY LEU ILE ASP PHE VAL IAP GLY ILE VAL GLY PRO SER  
451 GLN IAP ASP ALA PHE LEU VAL GLN ILE GLU GLN LEU ILE ASN GLU ARG ILE ALA GLU PHE ALA ARG ASN ALA ALA ILE ALA ASN LEU GLU  
541 GLN LEU GLY ASN ASN PHE ASN ILE TYR VAL GLU VAL ALA PHE LYS GLU IAP GLU ASP PRO ASN ASN PRO ALA THR ARG THR ARG VAL ILE  
631 ASP ARG PHE ARG ILE LEU ASP GLY LEU LEU GLU ARG ASP ILE PRO SER PHE ARG ILE SER GLY PHE GLU VAL PRO LEU LEU SER VAL TYR  
721 ALA GLN ALA ALA ASN LEU HIS LEU ALA ILE LEU ARG ASP SER VAL ILE PHE GLY GLU ARG IAP GLY LEU THR ILE ASN VAL ASN GLU  
811 CYS TYR ASN ARG LEU ILE ARG HIS ILE ASP GLU TYR ALA ASP HIS CYS ALA ASN THR TYR ASN ARG GLY LEU ASN ASN LEU PRO LYS SER  
901 THR TYR GLN ASP IAP ILE THR TYR ASN ARG LEU ARG ARG ASP LEU THR LEU THR VAL LEU ASP ILE ALA ALA PHE PHE PRO ASN TYR ASP



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ASN ARG ARG TYR PRO ILE GLN PRO VAL GLY GLN LEU THR ARG GLU VAL TYR ASP PRO LEU ILE ASN PHE ASN PRO GLN LEU GLN SER  
 VAL ALA GLN LEU PRO THR PHE ASN VAL MET GLU SER SER ALA ILE ARG ASN PRO HIS LEU PHE ASP ILE LEU ASN ASN LEU THR ILE PHE  
 THR ASP TAP PHE SER VAL GLY ARG ASN PHE TYR IAP GLY GLY HIS ARG VAL ILE SER SER LEU ILE GLY GLY ASN ILE THR SER PRO  
 ILE TYR GLY ARG GLU ALA ASN GLN GLU PRO PRO ARG SER PHE THR PHE ASN GLY PRO VAL PHE ARG THR LEU SER ILE PRO THR LEU ARG  
 LEU LEU GLN PRO CYS GLN ARG HIS PHE ASN LEU ARG GLY GLY VAL GLU PHE SER THR PRO THR ASN SER PHE THR TYR  
 ARG GLY ARG GLY THR VAL ASP SER LEU THR GLU LEU PRO PRO GLU ASP ASN SER VAL PRO PRO ARG GLU GLY TYR SER HIS ARG LEU CYS  
 HIS ALA THR PHE VAL GLN ARG SER GLY THR PRO PHE LEU THR THR GLY VAL VAL PHE SER TAP THR HIS ARG SER ALA THR LEU THR ASN  
 THR ILE ASP PRO GLU ARG ILE ASN GLN ILE PRO LEU VAL LYS GLY PHE ARG VAL TAP GLY THR SER VAL ILE THR GLY PRO GLY PHE  
 THR GLY GLY ASP ILE LEU ARG ARG ASN THR PHE GLY ASP PHE VAL SER LEU GLN VAL ASN ILE ASN SER PRO ILE THR GLN ARG TYR ARG  
 LEU ARG PHE ARG TYR ALA SER SER ARG ASP ALA ARG VAL ILE VAL LEU THR GLY ALA SER THR GLY VAL GLY GLN VAL SER VAL

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1101 ALA MET PRO LEU GLN LYS THR MET GLU ILE GLY, GLU ASN LEU THR SER ARG THR PHE ARG TYR THR ASP PHE SER, ASN PRO PHE SER PHE  
 1201 PHE ALA ASN PRO ASP ILE ILE GLY ILE SER GLU GLN PRO LEU PHE GLY ALA GLY SER ILE SER SER GLY GLU LEU TYR ILE ASP LYS ILE  
 2071 GLU ILE ILE LEU ALA ASP ALA THR PHE GLU ALA, GLU SER ASP LEU GLU ARG ALA GLN LYS ALA VAL ASN ALA LEU PHE THR SER SER ASN  
 2101 GLN ILE GLY LEU LYS THR ASP VAL THR ASP TYR, HIS ILE ASP GLN VAL SER ASN LEU VAL ASP CYS LEU SER ASP GLU PHE CYS LEU ASP  
 2231 GLU LYS ARG GLU LEU SER GLU LYS VAL LYS HIS ALA LYS ARG LEU SER ASP, GLU ARG ASN LEU LEU GLN ASP PRO ASN PHE ARG GLY ILE  
 2341 ASN ARG GLN PRO ASP ARG GLY TRP ARG GLY SER THR ASP ILE THR ILE GLN GLY GLY ASP VAL PHE LYS GLU ASN TYR VAL THR LEU  
 2431 PRO GLY THR VAL ASP GLU CYS TYR PRO THR TYR LEU TYR GLN LYS ILE ASP GLU SER LYS LEU LYS ALA TYR THR ARG TYR GLU LEU ARG  
 2521 GLY TYR ILE GLU ASP SER GLN ASP LEU GLU ILE TYR LEU ILE ALA TYR ASN ALA LYS HIS GLU ILE VAL ASN VAL PRO GLY THR GLY SER  
 2611 LEU TRP PRO LEU SER ALA GLN SER PRO ILE GLY LYS CYS GLY GLU PRO ASN ARG CYS ALA PRO HIS LEU GLU TRP ASN PRO ASP LEU ASP  
 2701 CYS SER CYS

15/ Recombinant expression and cloning vector containing at least a part of the nucleotide sequence such as defined in any one of the Claims 1 to 14.

16/ Plasmid according to Claim 15 characterized in that it is pHT671 as represented in figure 4, or pHT71 comprising a HindIII-PstI DNA fragment constituted uniquely of DNA derived from the afzawai 7-29 strain.

17/ Modified bacterial strains, characterized in that after transformation they contain a sequence of nucleotides according to one of the Claims 1 to 14.

18/ Bacterial strain according to Claim 17, characterized in that it contains at least one recombinant vector according to Claim 15 or 16.

19/ Polypeptide toxic towards larvae of the Lepidoptera and preferentially towards S.littoralis, characterized in that it is capable of forming an immunological complex with antibodies directed against polypeptides with larvicidal activity towards S.littoralis.

20/ Polypeptide according to Claim 19, characterized in that it contains the sequence (II) or the sequence (IV) of amino acids defined in Claim 14.

21/ Procedure for obtaining a nucleotide sequence coding for at least a part of the N-terminal region of a polypeptide toxic specifically towards larvae of the Lepidoptera of the family of the Noctuidae, and preferentially towards S.littoralis, characterized by the following steps :

- the carrying out of a hybridization between a sequence of nucleotides from a strain of B.thuringiensis active against S.littoralis, on the one hand, and, on the other, one or several sequences of nucleotides utilized as probes derived from the 5' part of a restriction fragment of a gene for a  $\delta$ -endotoxin of B.thuringiensis, this part coding for the N-terminal part of a polypeptide toxic towards the Lepidoptera, and derived from the 3' part of this fragment coding for the COOH part of the polypeptide,
- the isolation of the fragment,
- its cloning in a vector, followed by its purification.

22/ Procedure according to Claim 21, characterized in that the hybridization probes utilized are obtained from a gene for a  $\delta$ -endo-toxin derived from a aizawai 7-29 strain coding for a protein of 130kDa active against P.brassicae and inactive towards S.littoralis, this gene having been cloned in the recombinant plasmid pHTA2.

23/ Procedure according to Claim 21 or 22, characterized in that the fragment recombined with the vector in the cloning step is elaborated from at least one sequence of nucleotides derived from at least one recombinant vector containing a sequence of nucleotides from at least one strain of B.thuringiensis.

24/ Procedure according to Claim 23, characterized in that the fragment recombined with the vector in the cloning step is elaborated from several sequences of nucleotides derived from recombinant vectors containing sequences of nucleotides from at least 2 different strains of B.thuringiensis, possessing the same restriction maps and themselves containing all or part of the sequences of nucleotides capable of coding for a polypeptide active preferentially towards S.littoralis.

25/ Procedure according to Claim 23, characterized in that the fragment recombined with the vector in the cloning step is elaborated from a HindIII-PstI restriction fragment derived from the aizawai 7-29 strain.

26/ Procedure according to Claim 24, characterized in that the fragment recombined with the vector in the cloning step is elaborated from a HindIII-HincII restriction fragment derived from the entomocidus 6-01 strain and from a HincII-PstI restriction fragment derived from the aizawai 7-29 strain.

27/ Procedure according to Claim 22, characterized in that the restriction fragment recombined according to Claim 25 is carried preferentially by a plasmid pHTA6 and the restriction fragments recombined according to Claim 26, HindIII-HincII and HincII-PstI, are carried preferentially by the respective recombinant plasmids pITE6 and pHTA6, the said plasmids pHTA6 and pITE6 being those isolated with the aid of a probe constituted by a PvuII fragment of 2 kb of the plasmid pBT15-38 corresponding to the internal part of a gene for the chromosomal crystal of the berliner 1715 strain, from

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transforming clones containing nucleotide sequences derived from B.thuringiensis strains active towards larvae of the Lepidoptera, inter-alia S.littoralis.

28/ Larvicidal composition with preferential activity towards  
5 S.littoralis, characterized in that it contains an efficacious amount  
of polypeptide such as defined in any one of the Claims 19 to 20  
expressed by the nucleotide sequences according to any one of the  
Claims 1 to 14, the vector according to the Claim 15, or the plasmid  
according to the Claim 16, or the bacterial strain according to any  
0 one of the Claims 17 or 18.

29/ Application of the nucleotide sequences according to any one of the Claims 1 to 14 to produce a polypeptide toxic towards Lepidoptera, and preferentially S.littoralis, in microorganisms capable of expressing recombinant vectors containing these sequences such as E.coli, B.subtilis, B.cereus or B.thuringiensis.

30/ Application according to Claim 29, characterized in that the sequences of nucleotides are introduced into microorganisms living in the environment or in association with the plants such as Pseudomonas, Azospirillum or Rhizobium and capable of expressing recombinant vectors containing these sequences.

31/ Application according to Claim 29 or 30, characterized in that the nucleotide sequences are introduced into microorganisms in combination with different  $\delta$ -endotoxin genes.

32/ Application of the nucleotide sequences according to any  
25 one of the Claims 1 to 14 to the transformation of plants sensitive  
to S.littoralis, characterized in that it comprises the transfer and  
the expression of these sequences in these plants.

30 33/ Plant cells, the genome of which, after transformation by means of a non-essentially biological procedure, possesses in a stable manner a sequence of nucleotides capable of expressing a polypeptide toxic towards S.littoralis, such as defined in any one of the Claims 1 to 14 and cells derived from their division.

34/ Plants having in particular S.littoralis as predator,  
transformed by a non-essentially biological procedure, the genome  
35 of which possesses in a stable manner a sequence of nucleotides such

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as defined in any one of the Claims 1 to 14, capable of expressing a polypeptide toxic towards S.littoralis and plants derived from their reproduction, their multiplication, or hybrid crosses.

35/ Plant having in particular S.littoralis as predator,  
possessing in addition to their initial phenotypic and genotypic  
characters the property of expressing a polypeptide toxic  
preferentially towards S.littoralis, this property resulting from  
the insertion in its genome by genetic manipulation of a sequence  
of nucleotides capable of expressing the said polypeptide.

10            36/            Seed capable of giving rise to a plant according to Claim  
34 or 35 or derived from such a plant, characterized in that it has  
integrated into its genome, by genetic manipulation, a sequence of  
nucleotides according to any one of the Claims 1 to 14.

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Washington, D. C.